

Unilateral Incompatibility in *Capsicum* (Solanaceae): Occurrence and Taxonomic Distribution

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• **Background and aims** Unilateral incompatibility (UI) occurs when pollinations between species are successful in one direction but not in the other. Self-incompatible (SI) species frequently show UI with genetically related, self-compatible (SC) species, as pollen of SI species is compatible on the SC pistil, but not vice versa. Many examples of unilateral incompatibility, and all those which have been studied most intensively, are found in the Solanaceae, particularly *Lycopersicon*, *Solanum*, *Nicotiana* and *Petunia*. The genus *Capsicum* is evolutionarily somewhat distant from *Lycopersicon* and *Solanum* and even further removed from *Nicotiana* and *Petunia*. Unilateral incompatibility has also been reported in *Capsicum*; however, this is the first comprehensive study of crosses between all readily available species in the genus.

• **Methods** All readily available (wild and domesticated) species in the genus are used as plant material, including the three genera from the *Capsicum pubescens* complex plus eight other species. Pollinations were made on pot-grown plants in a glasshouse. The number of pistils pollinated per cross varied (from five to 40 pistils per plant), depending on the numbers of flowers available. Pistils were collected 24 h after pollination and fixed for 3–24 h. After staining, pistils were mounted in a drop of stain, squashed gently under a cover slip and examined microscopically under ultra-violet light for pollen tube growth.

• **Key results** Unilateral incompatibility is confirmed in the *C. pubescens* complex. Its direction conforms to that predominant in the Solanaceae and other families, i.e. pistils of self-incompatible species, or self-compatible taxa closely related to self-incompatible species, inhibit pollen tubes of self-compatible species.

• **Conclusions** Unilateral incompatibility in *Capsicum* does not seem to have arisen to prevent introgression of self-compatibility into self-incompatible taxa, but as a by-product of divergence of the *C. pubescens* complex from the remainder of the genus.

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Key words: *Capsicum pubescens* complex, chile pepper, incompatibility, incongruity, interspecific hybridization, pollen tube growth, *S* gene.

INTRODUCTION

Unilateral incompatibility (UI) occurs when pollen tubes reach and fertilize the ovules in a cross made in one direction, but are inhibited in the stigma, style or ovary in the reciprocal cross. It has most often been reported in the Solanaceae (Lewis and Crowe, 1958; Martin, 1963, 1967) and is often associated with differences in self-compatibility between the taxa being crossed.

Unilateral incompatibility may occur between genera: for example, when stigmas of *Capsicum frutescens* (self-compatible) are pollinated by self-incompatible accessions of *Lycopersicon hirsutum* or *L. peruvianum*, the *Lycopersicon* pollen tubes reach the *Capsicum* ovaries, but in the reciprocal crosses, the *Capsicum* pollen tubes are <0.1 mm long (Lewis and Crowe, 1958). However, unilateral incompatibility is more commonly reported between species or groups of species within a genus: for example, self-compatible species in the *L. esculentum* complex will cross with the characteristically self-incompatible species *L. hirsutum* and *Lycopersicon peruvianum* only when used as female parents. In the reciprocal crosses, pollen tubes of the *L. esculentum* complex are inhibited in the upper portion

of the styles (Martin, 1967). Unilateral incompatibility may also occur within species: for example, pollen tubes of a northern race of *L. hirsutum* (which, unusually for this species, is self-compatible) are inhibited in the styles of most other accessions of *L. hirsutum* (SI), although the reciprocal crosses set fruit (Martin, 1963).

This association between self-incompatibility and unilateral incompatibility, together, with the observation that pollen tubes are often inhibited at approximately the same site, just below the stigma, in both self-incompatible and unilaterally incompatible crosses, has led to repeated suggestions that unilateral incompatibility is a pleiotropic effect of the *S* gene that controls self-incompatibility (Lewis and Crowe, 1958; Pandey, 1981; Chetelat and DeVerna, 1991; Bernacchi and Tanksley, 1997). However, there are a significant number of exceptions to the UI 'rule' that pistils of self-incompatible taxa inhibit pollen from self-compatible taxa. For example, unilateral incompatibility has been reported in crosses between some self-compatible species. Lewis and Crowe (1958) reviewed all such examples known to them and concluded that, in each case, one of the unilaterally incompatible species had close relatives which were self-incompatible, so had presumably mutated very recently from self-incompatibility to self-compatibility.

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Lewis and Crowe (1958) suggested that this mutation did not affect the response of the style to the pollen tube but rather inactivated the *S* allele in the pollen, enabling the pollen tube to grow down its own style (as well as down styles of other self-compatible species and self-incompatible species).

Subsequently, instances of unilateral incompatibility were also reported between self-incompatible taxa, and some reversals of the 'rule' were discovered, in which pistils of self-compatible taxa inhibited pollen from self-incompatible taxa, although the reciprocal crosses succeeded. These examples have been reviewed by de Nettancourt (1977, 2001) and it has been argued that these exceptions to the 'rule' invalidate the suggestions that the *S* gene controls unilateral as well as self-incompatibility. Hogenboom (1975) considered that the interspecific interaction of pollen and pistil involves successive barriers in the pistil, from stigma to ovule, each of which must be overcome if the male gametophyte is to penetrate the ovule. Whereas self-incompatibility may be controlled by a single gene, *S*, successive barriers to interspecific barriers to interspecific fertilization are likely each to be controlled by different genes.

As stated above, in solanaceous plants, SI is controlled by a single multiallelic locus, and *S* allele specific pollen rejection occurs as pollen tubes grow through down the transmitting tract. The products of the *S* locus in the style are the *S*-RNases (Murfett *et al.*, 1996). In some of the recent studies *S*-RNases have been also implicated in interspecific pollen rejection. In this manner; Kondo *et al.* (2002) studied the molecular basis of loss of self-incompatibility in genus *Lycopersicon*. In their study, *S*-RNase and HT-proteins were analysed in seven SC and three SI taxa. No, or low stylar *RNase* activity was reported in most SC taxa they examined, while high level of *RNase* activity was present in all SI species. They reported that the *S*-RNase gene was most likely deleted in the SC species of *L. esculentum*, *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium* and *L. cheesmanii* since there was no amplification of *S*-RNase genes from genomic DNA. However, the *S*-RNase gene was amplified from the genomes of SC species *L. chmielewski* and *L. hirsutum* f. *glabratum* as these species showed a decreased accumulation of transcripts. *S*-RNase and interspecific pollen rejection were also studied in other genera, e.g. *Nicotiana* (Murfett *et al.*, 1996), and the *S*-RNase mechanism was recently reviewed in detail by Cruz-Garcia *et al.* (2003).

Martin (1963) suggested that a unilateral incompatibility response may be brought about by different causes, and hence it may be under different controls, in different crosses. For example, it may sometimes result from divergence between genetically (often geographically) isolated taxa. This theory is basically similar to Hogenboom's concept of incongruity (Hogenboom, 1975). In other crosses (including those crosses that obey the unilateral incompatibility 'rule'), Martin (1963) suggested that unilateral incompatibility could have arisen through natural selection for barriers to prevent introgression of alleles for self-compatibility into outbreeding taxa which would suffer from inbreeding depression if selfed, as originally outlined Grun and Radlow (1961).

As mentioned above, many examples of unilateral incompatibility, and all those which have been studied most intensively, come from the Solanaceae, particularly *Lycopersicon*, *Solanum*, *Nicotiana* and *Petunia*. In contrast, the genus *Capsicum* has received little attention with regard to the occurrence of UI. *Capsicum* is evolutionarily somewhat distant from *Lycopersicon* and *Solanum* and even further removed from *Nicotiana* and *Petunia* (Olmstead and Palmer, 1992).

In *Capsicum*, unlike *Lycopersicon*, *Solanum* and *Nicotiana*, most wild species are self-compatible. Self-incompatibility is characteristic only of *Capsicum cardenasii*, which appears to be uniformly self-incompatible throughout its limited range in Bolivia (Yaqub and Smith, 1971). This species is closely related to, and may be sympatric with, *Capsicum eximium* (Esgbaugh, 1979). On the basis of chromosome structure (Pickersgill, 1991), isozyme banding patterns (McLeod *et al.*, 1983), flavonoid composition (Ballard *et al.*, 1970) and chloroplast DNA polymorphisms (Choong, 1998), *C. eximium*, *C. cardenasii* and domesticated *Capsicum pubescens* form a natural group within *Capsicum*. A second group consist of the *Capsicum annuum* complex, containing three domesticates (*C. annuum*, *Capsicum chinense* and *Capsicum frutescens*) and their conspecific wild relatives (Pickersgill *et al.*, 1979). The remaining domesticate, *C. baccatum*, with its conspecific wild relative and the closely related *Capsicum praetermissum* (McLeod *et al.*, 1983) constitutes a third group. The other wild species included in the study are *Capsicum chacoense* (native to Paraguay, Argentina and Bolivia), *Capsicum galapagoense* (endemic to Galapagos Islands) and *Capsicum tovarii* (from the inter-Andean valleys of central Peru). All three are distinct, well-defined species, but *C. tovarii* is morphologically most similar to, though isozymically distinct from, the *C. pubescens* complex (McLeod *et al.*, 1983), while *C. galapagoense* and *C. chacoense* are morphologically most similar to the *C. annuum* complex.

Choong (1998) working phylogenetic relationships of *Capsicum* by using molecular evidence from the chloroplast genome and the nuclear genome stated that cp DNA variation was distinctly low and the cpDNA phylogeny of *Capsicum* species in general agreed with relationships and groupings based on morphology and isoenzymes as stated above. Data obtained from cpDNA revealed that, while *C. annuum*, *C. chinense*, *C. frutescens* and *C. galapagoense* formed the *annuum* clade and *C. baccatum* and *C. praetermissum* formed the *baccatum* clade. *Capsicum chaconse* also formed a weakly supported sister group to the *baccatum* complex. The other species *C. eximium*, *C. cardenasii* and *C. pubescens* formed the *pubescens* clade as *C. pubescens* was basal to the *pubescens* clade. cpDNA data also revealed that *C. tovarii* was not related to the *C. pubescens* complex. Choong (1998) concluded that the unresolved basal internodes of the cp DNA tree might suggest that chile peppers had undergone evolutionary radiation and speciation.

Unilateral incompatibility has been reported in *Capsicum* (Pickersgill, 1991, 1997; Bermawie and Pickersgill, 1992; Zijlstra *et al.*, 1991) but this is the first study aimed to carry

TABLE 1. *Accessions used in this study*

Species	Status	Accession number	Breeding behaviour	Origin
<i>C. annuum</i>	Wild	BP 225	SC	Tabasco, Mexico
	Domesticated	C70-7a	SC	Mexico city, Mexico
<i>C. baccatum</i>	Wild	C 255	SC	Belo Horizonte, Brazil
	Domesticated	SA 219	SC	Viçosa, Brazil
<i>C. cardenasii</i>	Wild	E 1812	SI	La Paz, Bolivia
		SA 268	SC	La Paz, Bolivia
<i>C. chacoense</i>	Wild	BP 281	SC	Villeta, Paraguay
		SA 184	SC	Cordoba, Argentina
<i>C. chinense</i>	Wild	BP 605	SC	St Lucia, West Indies
	Semi-domesticated	C 248	SC	Patate, Ecuador
	Domesticated	C 334	SC	Bogota, Colombia
<i>C. eximium</i>	Wild	Hawkes 3860	SC	Sta. Victoria, Argentina
<i>C. frutescens</i>	Wild	Heiser 6240	SC	Guayaguil, Ecuador
	Semi-domesticated	SA 36	SC	Sincelejo, Colombia
<i>C. galapagoense</i>	Wild	AC 1501	SC	Galapagos Is., Ecuador
<i>C. praetermissum</i>	Wild	C 343	SC	Sao Paulo, Brazil
<i>C. pubescens</i>	Domesticated	BP 43	SC	Cuzco, Peru
		BP 537	SC	Cuzco, Peru
<i>C. tovarii</i>	Wild	BP 382	SC	Ayacucho, Peru

SC, Self-compatible; SI, self-incompatible.

out the comprehensive investigation of the occurrence of UI within the genus *Capsicum* by making the crosses between all readily available species in the genus.

MATERIALS AND METHODS

Plant material

Eleven of the most commonly recognized species (out of 27) of wild and domesticated forms (accessions) of some species (a total of 19 different accessions) were used as plant material in this study. The accessions used are listed in Table 1. Of the two accessions of *C. cardenasii*, SA 268 was collected in 1959 and was then self-incompatible. Possibly as a result of inadvertent selection for spontaneous fruit set in our glasshouse, the stock of this accession is now self-compatible. Accession E 1812 was collected more recently and is self-incompatible.

Hand pollinations

All pollinations were made on pot-grown plants in a glasshouse. Buds, prior to anthesis, were emasculated in the morning or early afternoon by removing the corolla and undehiscent stamens with fine forceps. The stigmas were then pollinated with pollen from a flower whose anther had dehisced that day. The stigmas were not covered in any way after pollination as it was decided that pollination by wind and insects was minimal, if there was any, since the plants were planted in protected enclosures and the absence of petals (the emasculation also removes the nectaries) made the pistils unattractive to insects.

Flowers of all species under study were selfed to determine the time taken by pollen tubes to reach the ovules. The flowers were harvested at the following time intervals: 4 h, 8 h, 24 h and 48 h. From the results of these self-pollinations it was decided to harvest the pistils 24 h after pollination.

Interspecific crosses were made in both directions between all species or accessions shown in Table 1. The number of pistils pollinated per cross varied (5–40 pistils) depending on the numbers of accessions used per species and flowers available.

Study of pollen tube growth

Pistils were collected 24 h after pollination and fixed for 3–24 h in 3 parts absolute ethanol : 1 part glacial acetic acid. The fixed pistils were softened and stained by a method modified from Martin (1959). After fixation the pistils were rinsed twice with distilled water and hydrolysed in 1 M NaOH for 2 h at room temperature, followed by 15 min at 60 °C. They were then stained, either for 2 h at room temperature or overnight at 4 °C, in a solution of 2 g methyl blue and 20 g K₃PO₄ dissolved in 1 L distilled water. The stained pistils were mounted in drop of stain, squashed gently under a cover slip and examined microscopically for pollen tube growth under ultra-violet light.

The pistil was divided into six regions as follows: 1, stigma; 2, top of the style; 3, upper half of the style, excluding region 2; 4, lower half of the style, excluding stylar base; 5, base of style; 6, ovary. The region reached by the pollen tubes in any given pistil was recorded, and growth classes were then calculated according to Hermesen *et al.* (1977). The pollen tube growth data were analysed as a completely randomized design and standard error of means were calculated using MSTAT-C software program (MSTAT-C, Michigan State University, Version 1.2).

RESULTS

Domestication does not appear to have affected interspecific interactions between pollen tubes and pistils, since wild and domesticated accessions of the same species behaved in the same way. In Table 2, all data pertaining to each pair of

TABLE 2. *Region of the pistil reached by the longest pollen tube averaged over all pollinations between each pair of species*

		<i>pubescens</i> complex						Others					
		<i>C. cardenasii</i>		<i>exim.</i>	<i>pub.</i>	<i>ann.</i>	<i>chin.</i>	<i>frut.</i>	<i>bacc.</i>	<i>praet.</i>	<i>chac.</i>	<i>galap.</i>	<i>tov.</i>
		SI	SC										
<i>pubescens</i> complex	<i>cardenasii</i>	SI		5.3 (10) ± 0.15	5.2 (10) ± 0.15	1.2 (20) ± 0.09	1.2 (20) ± 0.09	1.2 (20) ± 0.09	1.2 (20) ± 0.11	1.4 (10) ± 0.16	1.2 (20) ± 0.09	1.2 (5) ± 0.30	1.5 (10) ± 0.16
		SC		5.3 (10) ± 0.15	5.3 (10) ± 0.15	1.2 (20) ± 0.09	1.2 (20) ± 0.09	1.2 (20) ± 0.09	1.4 (20) ± 0.09	1.2 (10) ± 0.13	1.2 (20) ± 0.09	1.0 (5) ± 0.00	1.6 (10) ± 0.16
Others	<i>eximium</i>				5.0 (20) ± 0.14	1.3 (20) ± 0.10	1.3 (20) ± 0.10	1.3 (20) ± 0.10	1.2 (10) ± 0.11	1.4 (10) ± 0.16	1.2 (20) ± 0.09	1.2 (5) ± 0.20	1.1 (10) ± 0.10
	<i>pubescens</i>			5.3 (10) ± 0.20	5.1 (20) ± 0.20	1.2 (40) ± 0.06	1.4 (40) ± 0.07	1.2 (40)	1.2 (20) ± 0.09	1.2 (20) ± 0.09	1.4 (40) ± 0.07	1.4 (5) ± 0.24	1.4 (10) ± 0.16
	<i>annuum</i>			5.6 (20) ± 0.11	5.6 (20) ± 0.11	5.8 (20) ± 0.09	5.1 (40) ± 0.06	5.6 (40) ± 0.07	5.6 (20) ± 0.11	5.6 (20) ± 0.11	5.5 (40) ± 0.08	5.4 (5) ± 0.20	5.7 (20) ± 0.10
	<i>chinense</i>			5.4 (20) ± 0.09	5.8 (20) ± 0.11	5.8 (10) ± 0.13	5.6 (40) ± 0.11	5.4 (40) ± 0.07	5.3 (10) ± 0.15	5.3 (10) ± 0.15	5.5 (40) ± 0.08	5.0 (5) ± 0.31	5.8 (20) ± 0.09
	<i>frutescens</i>			5.7 (20) ± 0.09	5.6 (20) ± 0.11	5.8 (20) ± 0.09	5.3 (40) ± 0.10	5.6 (40) ± 0.07	5.2 (20) ± 0.06	5.2 (20) ± 0.07	5.6 (40) ± 0.07	5.4 (5) ± 0.24	5.7 (20) ± 0.09
	<i>baccatum</i>			5.8 (40) ± 0.06	5.6 (40) ± 0.07	5.8 (20) ± 0.09	5.1 (40) ± 0.10	5.4 (40) ± 0.07	5.3 (40) ± 0.07	5.7 (20) ± 0.09	5.0 (40) ± 0.07	5.2 (5) ± 0.37	5.9 (20) ± 0.09
	<i>praeternisum</i>			5.8 (10) ± 0.13	5.8 (10) ± 0.13	6.0 (10) ± 0.00	5.0 (20) ± 0.10	5.8 (20) ± 0.09	5.4 (40) ± 0.11	5.4 (20) ± 0.11	5.8 (20) ± 0.09	5.2 (5) ± 0.20	5.9 (20) ± 0.06
	<i>chacoense</i>			5.8 (20) ± 0.09	5.8 (20) ± 0.09	5.6 (20) ± 0.11	5.4 (20) ± 0.11	5.6 (40) ± 0.07	5.8 (20) ± 0.06	5.6 (20) ± 0.11	5.8 (20) ± 0.09	5.2 (5) ± 0.37	5.4 (20) ± 0.10
	<i>galapagoense</i>			5.4 (5) ± 0.24	5.6 (5) ± 0.24	6.0 (5) ± 0.00	5.2 (5) ± 0.20	6.0 (5) ± 0.00	6.0 (5) ± 0.00	6.0 (5) ± 0.00	5.4 (5) ± 0.24	5.2 (5) ± 0.37	6.0 (5) ± 0.00
	<i>toarii</i>			5.0 (10) ± 0.00	5.6 (10) ± 0.16	5.6 (10) ± 0.16	5.0 (30) ± 0.10	5.8 (20) ± 0.11	5.6 (10) ± 0.09	5.7 (10) ± 0.15	5.7 (20) ± 0.09	5.6 (5) ± 0.24	

The values are given ± s.e.m. The number of pistils pollinated is in parenthesis. Figures in bold are unilaterally incompatible crosses.

species, with the exception of *C. cardenasii*, have therefore been averaged. For *C. cardenasii*, data on the self-incompatible (SI) and self-compatible (SC) accession are reported separately.

Crosses among species in the C. pubescens complex

The three species in this complex were bilaterally compatible in all combinations. Twenty-four hours after pollination, pollen tubes had reached the base of the style (region 5) in all pistils and had reached the ovules (region 6) in some pistils. The results were the same whether or not active *S* alleles were present in pollen or pistil, as self-compatible and self-incompatible accessions of *C. cardenasii* behaved in the same way.

Crosses between the C. pubescens complex and other species of Capsicum

Crosses between the three species in the *C. pubescens* complex and all other species included in this study showed unilateral incompatibility (data in bold in Table 2). Pollen tubes from species in the *C. pubescens* complex grew normally through the stigmas and styles of the other species and reached the bases of styles or ovules within 24 h. However, pistils of species in the *C. pubescens* complex inhibited pollen tubes of all other species in or just below the stigma.

Although *C. tovarii* is morphologically similar to species in the *C. pubescens* complex, it did not behave like the other species of this complex. It resembled the wild species outside the *C. pubescens* complex in showing unilateral incompatibility with the *C. pubescens* complex.

Crosses among species outside the C. pubescens complex

All pollinations between species outside the *C. pubescens* complex resulted in pollen tubes reaching the base of the style or the ovules within 24 h. No incompatibilities, unilateral or otherwise, were found within this diverse group of species. Pollinations involving the morphologically and isozymically distinct *C. tovarii*, or the geographically isolated *C. galapagoense*, resulted in pollen tubes growing as fast and as far as (or farther than) pollen tubes in pollinations among the very closely related members of the *C. annuum* complex or the *C. baccatum* complex.

DISCUSSION

Unilateral incompatibility in *Capsicum* conforms to the classic pattern discussed by Lewis and Crowe (1958). It is associated with the only group of species in the genus *Capsicum* in which self-incompatibility is known to occur, namely the *C. pubescens* complex. Pistils of species in the *C. pubescens* complex inhibit pollen tubes of species outside the *C. pubescens* complex, while the reciprocal crosses are compatible.

The unilateral incompatibility shown in this study for the self-compatible *C. eximium* or *C. pubescens* and the species outside the *C. pubescens* complex, add to the already known

examples of unilateral incompatibility between pairs of self-compatible species. According to Lewis and Crowe's hypothesis (Lewis and Crowe, 1958), *C. eximium* and *C. pubescens* are assumed to be derived from self-incompatible ancestors and to have developed self-compatibility relatively recently. In many groups of flowering plants, including many genera of Solanaceae, self-compatibility does indeed appear to be derived from self-incompatibility (e.g. Kondo *et al.*, 2002), but this is less plausible in the case of *Capsicum*. In *Capsicum*, unlike *Solanum*, *Lycopersicon* or *Nicotiana*, self-compatibility is the norm. Self-incompatibility is characteristic only of *C. cardenasii*, a species restricted to a small area of north-east Bolivia. The chloroplast DNA phylogeny (Choong, 1998) shows *C. cardenasii*, together with *C. eximium*, as a sister group to *C. pubescens*. Self-incompatibility has been reported from a single accession of *C. pubescens* as well as *C. cardenasii* (Yaqub and Smith, 1971), so could be an ancestral state within the lineage of the *C. pubescens* complex. However, the single self-incompatible accessions of *C. pubescens* came from a site within the range of *C. cardenasii*. Another accession of *C. pubescens* collected from the same site had unusually small fruit and soft flesh (P. G. Smith, unpublished field notes), which are characteristics of wild peppers. It is thus possible that, at this locality, *C. pubescens* had hybridized with *C. cardenasii*, thereby acquiring small fruit, soft flesh and self-incompatibility. Further investigation of whether self-incompatibility really exists in 'pure' *C. pubescens* is therefore needed before self-compatibility in *C. eximium* and the majority of accessions of *C. pubescens* can be accepted as a derived state.

The geographic distributions of the wild species of *Capsicum* make it difficult to picture unilateral incompatibility originating as a device to prevent introgression of self-compatibility into a self-incompatible taxon, as suggested for *Solanum* by Grun and Radlow (1961) and for unilateral incompatibility in general by Abdalla and Hermesen (1972). *Capsicum cardenasii* is currently geographically isolated from all the wild species with which it is unilaterally incompatible. The self-compatible species with which it is most likely to introgress are wild *C. eximium* and the domesticated *C. pubescens* (Eshbaugh, 1979), with both of which it is bilaterally compatible. Moreover, no deleterious effects of inbreeding have been observed in *C. cardenasii*, even though both our accessions have been maintained as very small populations (no more than five plants in any given year) for many generations. It therefore seems to us more probable that, in *Capsicum*, unilateral incompatibility has arisen as a by-product of genetic divergence between the *C. pubescens* complex and the other chile peppers, not as a product of natural selection.

Unilateral incompatibility has proved a more reliable guide than morphology in assessing the relationships of *C. tovarii*. When first described, this species was assumed to be related to the *C. pubescens* complex (Eshbaugh *et al.*, 1983), although it was genetically distant from this complex and from all other species of *Capsicum* on the basis of isozyme alleles (McLeod *et al.*, 1983) and cpDNA data (Choong, 1998). Our data on interspecific pollinations confirm and extend those of Bermawie and Pickersgill (1992).

Capsicum tovarii is bilaterally compatible with all species outside the *C. pubescens* complex and resembles those species in showing unilateral incompatibility with the *C. pubescens* complex. We therefore consider that *Capsicum tovarii* should not be included in the *C. pubescens* complex.

The mechanism and genetic control of unilateral incompatibility in *Capsicum* remain unclear. Unilateral incompatibility is expressed in the stigma or uppermost part of the style in *Capsicum*, whereas Yaqub and Smith (1971) found that self-incompatible pollen tubes could penetrate one-fifth of the style of *C. cardenasii*. Others have likewise found that unilateral incompatibility in the Solanaceae results in an earlier inhibition of the pollen tubes than does the self-incompatibility response (Lewis and Crowe, 1958; Martin, 1963; Mutschler and Liedl, 1994), but interpretations have differed in this respect. Lewis and Crowe (1958) considered that both phenomena are controlled by the *S* gene; Mutschler and Liedl (1994) regarded their data as evidence that unilateral incompatibility acts before the self-incompatibility system comes into effect and considered furthermore that the two phenomena are not functionally related.

Bud pollination has been used successfully to overcome both unilateral incompatibility (Gradziel and Robinson, 1989, 1991) and self-incompatibility (Herrero and Dickinson, 1980) in Solanaceae. In the case of self-incompatibility, this has been correlated with the time of appearance of the *S* gene product in the pistil (Clarke *et al.*, 1985), but this does not necessarily implicate the *S* gene product in unilateral incompatibility. Since the argument about the relationship, if any, between self-incompatibility and unilateral incompatibility has not been resolved even in intensively investigated crosses within and between species of *Lycopersicon* and *Solanum*, it is hardly surprising that our data from much less intensively studied crosses in *Capsicum* can be interpreted in different ways.

Further studies, such as expression of *S-RNase*-like genes in white- and purple-flowered taxa, in *Capsicum* would definitely help to determine the cause of unilateral incompatibility in the genus.

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